## Protocols

## Annealing Buffer

1. Add 0.2ml of 0.5M EDTA, 5ml of 1M NaCl, and 1ml of 1M tris-HCl.

2. Calibrate pH meter with appropriate buffers

3. Measure pH of buffer mixture while stirring (add water if necessary).

4. The desired pH range is 7.5-8. To acheive this add 1M NaOH 100µl at a time.

5. Once at desired pH make solution up to 100ml using dH2O.

## Annealing of primers

1) Spin primers (dried)
2) Add buffer required by IDT sheet to make 100 µM.
3) Vortex and spin
4) Add 48.6 µl of TE annealing buffer to make up to 50 µl.
5) Add 0.7 µl of each primer to the buffer
6) Spin
7) Heat for 2 min at 92˚C on heating block and then at room temperature to cool down slowly
8) If using Fluorescent primers must keep shielded from light as much as possible.

## Antibiotic preparation

Antibiotic Solutions

Table1

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | | **Stock Solution*** | **Working concentration** | | |
| 2 | | **Concentration** | **Storage** | **Stringent plasmids** | **Relaxed Plasmids** |
| 3 | **Ampicillin** | 50 mg/ml in H2O | -20C | 20 µg/ml | 60 µg/ml |
| 4 | **Carbenicillin** | 50 mg/ml in H2O | -20C | 20 µg/ml | 60 µg/ml |
| 5 | **Chloramphenicol** | 34 mg/ml in ethanol | -20C | 25 µg/ml | 170 µg/ml |
| 6 | **Kanamycin** | 10 mg/ml in H2O | -20C | 10 µg/ml | 50 µg/ml |
| 7 | **Streptomycin** | 10 mg/ml in H2O | -20C | 10 µg/ml | 50 µg/ml |
| 8 | **Tetracycline**** | 5 mg/ml in ethanol | -20C | 10 µg/ml | 50 µg/ml |
| 9 | | | | | |

## Bacterial Glycerol Stock

-Take 1ml of overnight culture;

-Add 500µl of 60% glycerol stock;

-Put at -80ºC.

**Beads-Anchor binding efficiency assay**

**Materials**

- magnetic rack
- beads solution
- anchor solution (10 µM)
- TE + NaCl buffer (annealing buffer)
- d. H2O

Execution:

Aliquotte 25 µl of beads into 8 eppindorf tubes and pellet them on the magnetic rack

Wash the beads with 100 µl of anneal buffer 2 times (add the washing buffer, shake to mix them and pellet beads on magnetic rack, then remove the supernatant and put the next washing solution) then repeat the same with 100 µl d. $H_2O$

Prepare a 8 series of diluted anchor solutions 2/3 dilution of the stock. Set up one control buffer solution.

Add 12.5 µl of each anchor solution to the bead-only tubes and shake the tubes to mix them well (This is to be done for 10 min at room temperature)

Pellet the beads and remove the supernatant, wash again as in step 2

Add 100 µl of anneal buffer to the beads and resuspend. Heat them at 50 C on a heat block for 5 min

One by one, pellet the beads with the rack while they are still hot and collect the supernatant for measurement. If they can't be pelleted before cooling, protocol has to be revised.

Beads can then be reused for further binding assays, using the same beads.

**Chemically Competent Cells**

<u>CaCl2 method</u>

- Inoculate a single colony of appropriate cells into 10 ml LB in a sterilin. Add antibiotic if needed, and culture o/n at 37°C, 200 rpm;
- Inoculate 100 ml LB with 1 ml o/n culture;
- Incubate at 37°C, 200 rpm until OD600=0.3-0.6 (~2hrs);
- Transfer to 2x50 ml Falcon tubes and leave on ice for 30 mins;
- Centrifuge at 4000 x g, 5 mins, 4°C;
- Re-suspend pellet GENTLY in 25 ml ice cold 0.1 M MgCl2;
- Incubate on ice for 30 mins;
- Centrifuge at 4000 x g, 5 mins, 4°C;
- Re-suspend pellet GENTLY in 25 ml ice cold 0.1 M CaCl2;
- Incubate on ice for 30 mins;
- Centrifuge at 4000 x g, 5 mins, 4°C;
- Re-suspend pellet GENTLY in 25 ml ice cold 0.1 M
- CaCl2/Glycerol solution (1.7 ml 0.1 M CaCl2, 0.3 ml 100% Glycerol);
- Aliquot (200 µl) and fresh freeze in liquid nitrogen.
- Store in -80 freezer.

| Solution | Concentration | Amount for 100 ml |
|---|---|---|
| MgCl2 | 0.1 M | to 100 ml |
| CaCl2 | 0.1 M | to 100 ml |
| Glycerol | 100 % (v/v) | 100 ml |
| Stir until dissolved. Filter salts with 0.22 µm bottle top filter, autoclave. | | |

## Digestion for Cloning

*Digestion to check clones*

- Mix and do digestion mixture:
- 2µl Buffer (appropriate for enzyme)
- 0.5-1µl Enzyme (s) (do this step LAST)
- DNA (about100-200ng) 2 µl of miniprep
- Fill Water till 20µl
- Leave for 2h at appropriate temperature;

- If not to use right away, freeze.

*Digestion of plasmid for cloning*

- Mix:
- 20µl Buffer (appropriate for enzyme)
- 2µl Enzyme (s)
- DNA (about1-2µg) Xµl
- Fill Water till 200 µl
- Leave for 2h at appropriate temperature;
- If not to use right away, freeze.

# Gel Electrophoresis

**0,7% Agarose Gel**

- Mix 0,7 g of agarose and 100 ml of 1x TAE;
- Heat up for 2 min;
- Leave to cool down;
- Add 5 µl of Ethidium Bromide (10mg/ml);
- Put on gel caster;
- Let it cool down and gelify;
- If not to use right away, store in fridge.

**Running Products**

- Put gel on running apparatus;
- Load 20 µL of ladder;
- Load samples (x amount sample + 6x loading buffer + $H_20$)
- Run 90 mV for 45 min.

**Loading buffer:**

- 333 µl of 60% glycerol
- 10µl of 0.5M EDTA
- 20-100µl 6xDye
- Complete till 1ml with $H_2O$

# Ligation

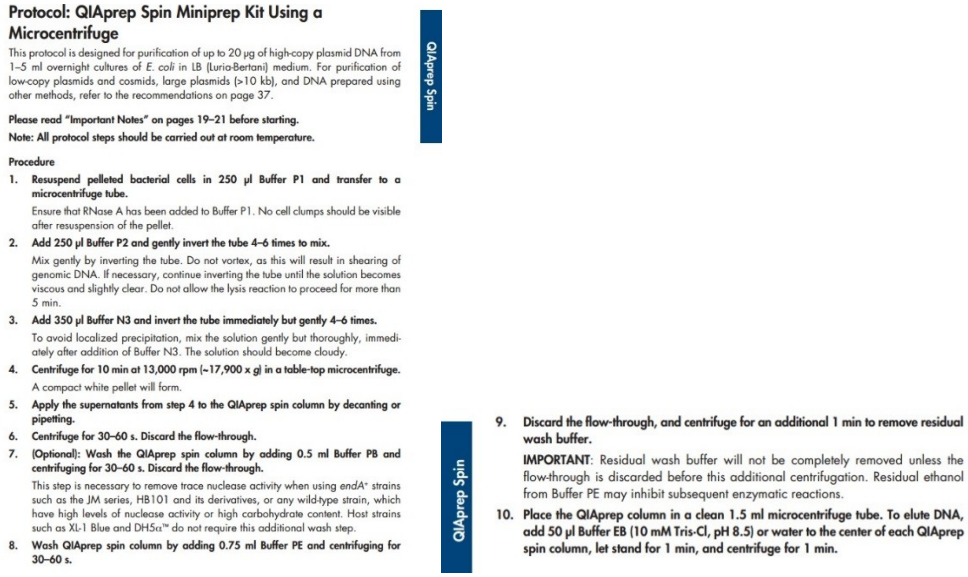
**Ligation**

DNA (Cleaned up digestion) 5µl
T4 DNA ligase buffer 2.5µl
T4 DNA ligase enz 0.5µl
Water 17µl
Incubate at room temperature for a minimum of 2hrs
Ligation can then be frozen and used another day. Even when transform always freeze remaining so can try another DNA/bacteria ratio if no colonies.

**Transformation**

This is done using the TOP 10 cells. Try with 100µl cells 2.5µl ligation. If this doesn't work then you can try more cells with more DNA, eg 50µl cells 7.5µl ligation.
[NOTE: When I have problem with a long primer due to needing that design I added gradient of DMSO to PCR. This was successful.]

**Miniprep**



**Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge**

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37.

**Please read "Important Notes" on pages 19–21 before starting.**

**Note: All protocol steps should be carried out at room temperature.**

QIAprep Spin

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**
   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
2. **Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix.**
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
3. **Add 350 µl Buffer N3 and invert the tube immediately but gently 4–6 times.**
   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.
4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.**
   A compact white pellet will form.
5. **Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.**
6. **Centrifuge for 30–60 s. Discard the flow-through.**
7. **(Optional): Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.
8. **Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**

QIAprep Spin

9. **Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**
   **IMPORTANT**: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. **Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

# Oligos

## "Oligos" golden rules

### PCR

21 mer;
(8) $9 \leq (G+C) \leq 11$;
Nice mix of GATCC (ie not GGGGG);
3' ends are not complementary;
No GC concentration near 3'.

### Sequencing

18 mer;
Nice mix of GATC;
$7 \leq (G+C) \leq 11$.

### Primer 3 settings

Size range (for dsRNA) 400-800;
Max 3' stabilization=7;
Primer size= 18bp;
Primer CG% between 40-60%;
Max sel-complementarity 6;

# PCR

**Volumes of each component needed for one 25 µl reaction (when preparing the mastermix always prepare for two reactions more, e.g.: 12 for 10 reactions)**

- MasterMix: 13.75 µl H2O, 5 µl buffer, 0.50 µl dNTPs, 2.50 µl DMSO
- In each sample tube add 1.25 µl of each primer on the right and left walls, and 0.50 µl of template DNA

- Add 0.25 µl of enzyme to the master mix
- Add mastermix to the sample tube

## Phosphorylation

- 50 µl of DNA

- 10 µl of 10x LIGASE buffer

- make up to 100 µl with H2O

- 0.5 µl of Kinase

Leave for 30 mins at room temperature

**Protocol for the assembly of a two words BabbleBlock**

1) Remove the beads from the fridge and shake the bottle to make sure the beads pellet is re-suspended.

2) Pour 20 µl of beads in a 1.5 ml Eppendorf tube. Locate the tube on the magnetic rack, wait for the beads to aggregate on the wall and then remove the liquid.

3) Wash the beads: wash the beads twice, each time using 100 µl of annealing buffer (wash buffer). To wash the beads, the tube is removed from the rack, 100 µl are added and the tube is gently flicked until all the beads are in the solution. Then the tube is placed back into the rack and the supernatant is removed.

4) In a new Eppendorf tube, 25 µl of anchor is combined with 30 µl of first AB word, plus 10 µl of annealing buffer, 10 µl of Quick Ligase buffer and 0.6 µl of Quick Ligase. The reaction is left to occur for 5 min on the bench.

5) The solution of anchor and first word is then added to the beads. The tube is the left on the bench for 10 minutes so that the anchor can bind to the beads. In order for the reaction to take place, the tube is gently flicked every two minutes to make sure all the beads are in the solution.

6) After the ten-minute gap, the tube is placed on the rack and the supernatant is removed.

7) The beads are washed like in step 3).

8) The next word is added as in step 4): 30 µl of BA word, 10 µl of annealing buffer, 10 µl of Quick Ligase buffer and 0.6 µl of Quick Ligase. The reaction is left for 5 minutes meanwhile the tube is gently flicked.

9) After adding the word, repeat step 6) and 7).

10) Step 8) is then repeated using the Terminator and is followed again by step 6) and 7).

11) 30 µl of Elution Buffer are then added to the beads. Flick the tube to make sure all the beads are in the solution, then place the tube on the incubator at 75°C for 10 min.

12) Place the tube on the rack and collect the supernatant.

## Resuspension of BioBricks

- use pipette to poke hole in the foil
- add 10 µl of dH2O
- pipette up and down
- leave to sit for 5 min
- transfer to PCR tube
- transform 1 µl of each

**Survival Assay**

- Grow E. Coli overnight in LB
- Dilute to 1A in 5ml LB-antibiotic
- Grow for __ hours
- Pellet cells
- Resuspend in 1ml saline solution
- Repeat 4-5
- Dilute to .02A
- Aliquot into plates
- Transfer 100ul of cell suspension into 900ul saline solution (non-irradiated control)
- Slama Jama
- After exposure, transfer 100ul into 900ul
- After final irradiation, for each dose do three serial dilutions
- Inoculate 10ul of each dilution in chlr-LB overnight (damage reporter kan-LB)

## Transformation using TOP10 cells

- Defrost cells on ice. Take 100ul of competent TOP10 cells
- Add 1ul of plasmid DNA
- Vortex and put on ice for 30min (turn on waterbath to 42*C and 37*C)
- Heat shock at 42*C in a waterbath for 30secs
- Return to ice for 30sec
- Add 1ml of SOC media
- Incubate at 37*C in waterbath for 1hr

- Plate 100ul on to the correct antibiotic plate
- Spin at 1000g the rest, remove most of media, reusupend in approx 100ul and plate onto another plate of the correct antibiotic
- Put plates to 37*C overnight, or on the bench over the weekend.

## Word-insertion protocol

**CIDAR MoClo Protocols**

| | Step | Temp | Time (min) |
|---|---|---|---|
| Standard protocol | Step 1 | 37°C | 1.5 |
| | Step 2 | 16°C | 3 |
| | Cycle 1-2 | x15 | |
| | Step 3 | 50°C | 5 |
| | Step 4 | 80°C | 10 |
| | Total time | | 82.5 |

| | | | |
|---|---|---|---|
| Troubleshooting protocol | Step 1 | 37°C | 1.5 |
| | Step 2 | 16°C | 3 |
| | Cycle 1-2 | x25 | |
| | Step 3 | 50°C | 5 |
| | Step 4 | 80°C | 10 |
| | Total time | | 127.5 |

**Reaction Conditions**

**Basic Part or Device (DVA Reactions)**

10 fmol each part
1x Promega Ligase buffer
20 U/rxn T4 Ligase (NEB or Promega)
10 U/rxn BbsI
Total Volume: 10 -20uL

**Transcriptional Unit (DVK Reactions)**

10 fmol each part
1x Promega Ligase buffer
20 U/rxn T4 Ligase (NEB or Promega)
10 U/rxn BsaI
Total Volume: 10 -20uL

Transform 2-5 uL per reaction